class II molecules and their known restriction patterns[15,16,18]. Moreover, peptides recognized in the context of the same restriction element could effectively compete with each other for *in vitro* binding and for functional recognition[18-20], suggesting that MHC class II molecules contain a single antigen-binding site. Similar experiments have not yet been reported for MHC class I molecules, but the demonstration that antigens recognized by class I restricted CTL can be mimicked by synthetic peptides[1,2], together with the presumptive structural homologies between class I and class II molecules[11,12], suggest that the general features involved in antigen recognition may be similar for both classes of MHC molecules. Further combinations of peptides, chimaeric class I MHC molecules, and CTL clones should be useful in resolving this issue.

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Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia

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Bacterial infection of the mammalian bloodstream can lead to overwhelming sepsis, a potentially fatal syndrome of irreversible cardiovascular collapse (shock) and critical organ failure. Cachectin, also known as tumour necrosis factor, is a macrophage-derived peptide hormone released in response to bacterial lipopolysaccharide, and it has been implicated as a principal mediator of endotoxic shock, although its function in bacterial sepsis is not known. Anaesthetized baboons were passively immunized against endogenous cachectin and subsequently infused with an LD_{100} dose of live *Escherichia coli*. Control animals (not immunized against cachectin) developed hypotension followed by lethal renal and pulmonary failure. Neutralizing monoclonal anti-cachectin antibody fragments ($F(ab')_2$) administered to baboons only one hour before bacterial challenge protected against shock, but did not prevent critical organ failure. Complete protection against shock, vital organ dysfunction, persistent stress hormone release and death was conferred by administration of antibodies 2 h before bacterial infusion. These results indicate that cachectin is a mediator of fatal bacteraemic shock, and suggest that antibodies against cachectin offer a potential therapy of life-threatening infection.

Sepsis can occur as a primary disease process or secondarily complicate other disease states, and is associated with a high mortality, despite antibiotic therapy[1,2]. Immunocompromised patients, such as those with malignancy, trauma or AIDS (acquired immune deficiency syndrome), are particularly susceptible. The toxicity of gram-negative bacteraemia was originally attributed to endotoxin/lipopolysaccharide (LPS), because many of the deleterious consequences of bacterial sepsis can be induced by LPS administration[3], but endogenous cytokines, rather than LPS itself, are now known to mediate the home static abn rmalities during endotoxaemia[4-7]. Serum levels of cachectin achieve nanomolar concentrations in experimental models of lethal endotoxaemia[7,8]: when infused into healthy animals, recombinant human-cachectin can induce haemodynamic collapse, counter-regulatory hormone release,

Table 1 Survival after pre-treatment with monoclonal anti-cachectin $F(ab')_2$ fragments during live gram-negative bacteraemia

Group	*n*	Survivors	Mean survival time (h ± s.e.m.)
Controls	6	0	13.3 ± 3.5
Antibody (−1 h)	3	0	17.4 ± 2.6
Antibody (−2 h)	3	3	sacrificed*

Female *Papio anubis* baboons (. .1 ± 0.6 kg) from the Charles River Primate Center (Port Jefferson, New York) were housed for a minimum of two weeks in the animal care facilities of the New York Hospital-Cornell University Medical Center (CUMC). The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at CUMC. Animals were free of infections or parasites, with haematocrits exceeding 36%. Food intake was restricted for 12 h before the experiment. Each baboon was immobilized with ketamine (14 mg kg^{-1}, i.m.) and the cephalic vein cannulated with a 20-gauge Teflon catheter; sodium pentobarbital was administered (2.0 mg kg^{-1} intravenously, at 30 min intervals) to maintain anaesthesia. The trachea was intubated with a cuffed tube and animals maintained spontaneous respirations on warming blankets. The superficial femoral vessels were exposed aseptically in one hindlimb; the artery was cannulated and connected to a pressure transducer used for the continuous monitoring of blood pressure and heart rate; the vein was cannulated with a flow-directed, thermodilution catheter (American Edwards Laboratories) which was guided into the pulmonary artery for measurement of pulmonary artery pressure and cardiac output. Control baboons were injected with carrier vehicle (5 ml 0.9% saline per kg, intravenous) by constant infusion over 30 min at $t = -1$ h ($n = 3$) or $t = -2$ h ($n = 3$). Experimental animals received anti-cachectin antibody $F(ab')_2$ fragments (10 mg $F(ab')_2$/kg in 5 ml 0.9% saline per kg) administered by constant intravenous infusion for 30 min beginning at $t = -1$ h or $t = -2$ h as indicated. Investigators were not informed whether infused carrier contained $F(ab')_2$. Lyophilized *E. coli* 086:B7 (from G. T. Shires) were used to inoculate slant cultures on tryptic soy broth agar[23]; viability counts of the inoculum were determined by standard dilution techniques. At time zero, baboons received an infusion of $1.2 \pm 0.3 \times 10^{11}$ live bacteria per kg body weight delivered into the aorta by constant infusion for 30 min; animals were maintained under anaesthesia and monitored continuously for 10 h, vital signs being recorded every 15 min, pulmonary artery pressures every 30 min, and cardiac output hourly.

* Animals surviving longer than 48 h were considered survivors and were subsequently sacrificed for necropsy at 2 d, 4 d, or 7 d.

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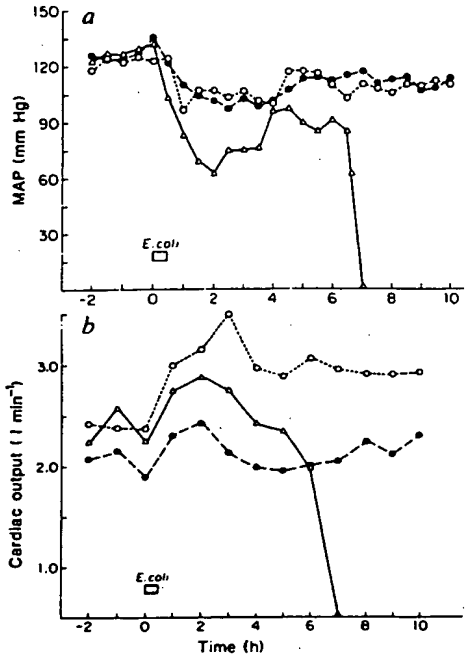

Fig. 1 *a*, Mean arterial blood pressure (MAP) and *b*, cardiac output in individual baboons during experimental primate bacteraemia. Intravenous *E. coli* was infused during the time indicated by the solid bars. The control (open triangle) received saline at $t = -2$ h; anti-cachectin $F(ab')_2$ was given at either $t = -1$ h (open circles) or $t = -2$ h (filled circles), as indicated. To more closely approximate the typical clinical resuscitation of septic human patients, and to minimize intravascular dehydration (which can confuse the interpretation of cardiovascular responses), Ringer's lactate solution was administered intravenously at a rate of 2 ml per kg-h throughout the study period. Additional Ringer's lactate was administered as a bolus (10 ml kg^{-1} over 15 min) if the pulmonary capillary wedge pressure (PCWP) fell below 2 mm Hg. PCWP was measured immediately after completion of the fluid bolus and the infusion repeated if necessary. Intravenous fluid administration was suspended if the PCWP was maintained at ≥3 mm Hg.

and critical organ injury in a pattern which is strikingly similar to that observed during lethal endotoxaemia[9,10]. Polyclonal antibodies raised against cachectin prevented death during subsequent endotoxaemia in mice, suggesting that cachectin might mediate lethal endotoxaemia[11]. Although LPS initiates the pathology of gram-negative sepsis, and cachectin can reproduce lethal endotoxaemia, it remains unclear whether cachectin is a necessary stimulus to septic shock.

In the present study, we produced lethal bacteraemia in baboons and specifically blocked endogenous cachectin by passive immunization with monoclonal anti-cachectin $F(ab')_2$ fragments. Anaesthetized baboons were monitored for blood pressure, pulmonary arterial pressure and cardiac output as described in the legend to Table 1, with one control and one $F(ab')_2$ antibody-treated animal studied simultaneously. Solutions were administered intravenously either 1 or 2 h before an LD_{100} dose of live *E. coli*[12,13], given by intra-aortic infusion. All animals surviving the 10-h study period were treated with an antibiotic (5 mg gentamicin kg^{-1} by intramuscular injection twice daily).

Maximal plasma cachectin levels (21.5 ± 3.8 ng ml^{-1}, mean ± s.e.m.) in the controls were observed between 1.5–2.5 h following the *E. coli* infusion, as assayed by a cachectin enzyme-linked immunosorbent assay (ELISA) (ref. 14). The concentration of serum cachectin returned from nanomolar to below detectable levels (34 pg ml^{-1}) 4–6 h after bacterial infusion; this rapid disappearance is consistent with previous observations during experimental endotoxaemia[8,14], and the magnitude of the response is sufficient to induce lethal shock[10]. In contrast, the administration of neutralizing anti-cachectin antibodies attenuated the circulating cachectin response (Table 2). The non-immunized (control) baboons rapidly succumbed to the lethal effects of gram-negative bacteraemia (Table 1). The infusion of live bacteria in the unprotected controls typically induced an acute decrease in both blood pressure and in pulmonary capillary wedge pressure, and an increase in heart rate (from 120 beats per min at baseline to >190 beats per min at 2 h) (Fig. 1). Cardiac output increased transiently with administration of intravenous fluid and in response to endogenous adrenaline release (Table 2). With the development of sepsis in controls, there was an inexorable decline in cardiac output, worsening shock, vital organ injury, and anuric renal failure associated

Table 2 Systemic hormone and leukocyte responses to live *E. coli* bacteraemia after pre-treatment with anti-cachectin $F(ab')_2$

	n	Sample time (h)*	Cachectin (ng ml^{-1})	Adrenaline (pg ml^{-1})	Noradrenaline (pg ml^{-1})	Glucagon (pg ml^{-1})	Leukocytes (×10^3 cells μl^{-1})
Controls	6	−2	<0.04	165 ± 27	382 ± 40	115 ± 17	13.2 ± 0.8
		2	16.7 ± 0.3	973 ± 302	2,344 ± 960	289 ± 34	1.1 ± 0.1
		8	<0.04	3,012 ± 861	3,411 ± 1,156	>1200	1.2 ± 0.1
Antibody (−1 h)	3	−2	<0.04	129 ± 26	958 ± 599	134 ± 48	9.3 ± 0.9
		2	1.2 ± 0.9	1,785 ± 1,135	2,591 ± 934	293 ± 92	1.0 ± 0.2
		8	<0.04	3,202 ± 1,087	3,934 ± 676	>1200	1.4 ± 0.2
Antibody (−2 h)	3	−2	<0.04	191 ± 25	419 ± 87	156 ± 16	12.1 ± 1.1
		2	<0.04	550 ± 162	491 ± 322	574 ± 14	3.6 ± 1.2
		8	<0.04	160 ± 16	372 ± 110	365 ± 123	8.2 ± 4.0

Murine monoclonal antibodies against recombinant human cachectin were produced in mouse ascites fluid and immunoglobulin (IgG) prepared by ammonium sulphate fractionation. The $F(ab')_2$ fragments were generated by pepsin digestion of IgG (10 mg ml^{-1}) with pepsin (0.02 mg ml^{-1}) in citrate buffer (*p*H 3.5) for 3 h at 37 °C (ref. 24). $F(ab')_2$ was purified by DEAE-Sepharose column chromatography and dialysed exhaustively against phosphate-buffered saline (*p*H 7.4). Neutralizing activity of the purified $F(ab')_2$ was determined in a standard bioassay based on L929-cell cytotoxicity[25,26]. Purified $F(ab')_2$ completely neutralized at least 50 ng ml^{-1} of recombinant human cachectin (10^8 U mg^{-1}) at an antibody concentration of 10 μg ml^{-1}. The purified $F(ab')_2$ (10 μg ml^{-1}) also completely neutralized L929 cell cytotoxicity of baboon plasma collected 2.5 h after bacteraemia during a maximal cachectin response (L929 cell cytotoxicity equivalent to 100 ng ml^{-1} r-human cachectin). The endotoxin/LPS content of the purified $F(ab')_2$ solution was less than 0.25 ng LPS per mg protein, as assayed by the Limulus amoebocyte lysate test. Baseline arterial blood was collected at $t = -2$ h after the pulmonary capillary wedge pressure had stabilized at 2–3 mm Hg. Samples were placed in ice, centrifuged (2,100 r.p.m., 4 °C, 20 min) and stored at −70 °C. Heparinized plasma was assayed for cachectin by ELISA (ref. 14); adrenaline and noradrenaline were assayed by radioenzymatic assay[27]; plasma for glucagon was collected with EDTA and aprotinin before analysis by radioimmunoassay[28]. Data recorded as mean ± standard error.

* Samples were collected at the following times: baseline specimens (before the infusion of antibody or saline) at $t = -2$ h; after the start of the bacterial infusion ($t = 2$ h); and $t = 8$ h, or immediately before death.

with a twofold increase in serum creatinine, followed by death from pulmonary oedema. These pathophysiological events are characteristic of lethal, hypodynamic sepsis[12,13].

Passive immunization of baboons with anti-cachectin antibody infused 1 h before bacterial challenge conferred beneficial cardiovascular effects (Fig. 1) but not complete protection against critical organ injury (Table 1). In contrast to the controls, blood pressure did not fall acutely after bacteraemia, but was maintained by a compensatory increase in heart rate (up to 175 beats per min) and cardiac output. Although acute cardiovascular collapse and shock were not observed, serious renal injury did ccur, as evidenced by twofold increases of serum creatinine at 8 h and the development of anuria. Each of the baboons immunized 1 h before bacterial challenge developed fatal pulmonary oedema.

Should uniform distribution and tissue penetration of the antibody not have occurred within 1 h, we investigated protection using earlier passive immunization with anti-cachectin $F(ab')_2$. When antibody was administered 2 h before *E. coli*, normal blood pressure was maintained during bacteraemia, significant tachycardia occurred only transiently, and shock was prevented. Neither did vital organ dysfunction occur or renal failure develop, and there was no evidence of pulmonary edema. Animals recovered from anaesthesia, were active and healthy, and resumed eating within 24 h. No evidence of persisting sepsis or tissue injury was observed by physical examination r r utine complete blood count until the time that animals were killed for necropsy. Thus, early passive immunization with mon cl nal anti-cachectin antibodies protected against the effects f bacterial sepsis.

These results with anti-cachectin antibodies were not due to increased bacterial clearance in the immunized animals, or to bacteri cidal properties of the antibody solution: the viability of circulating bacteria 2 h after *E. coli* infusion was similar in the contr l ($3.1 \pm 0.5 \times 10^4$ CFU per ml) and immunized baboons ($3.9 \pm 0.8 \times 10^4$ CFU per ml), and decreased comparably in both groups within 4 h ($1.3 \pm 0.6 \times 10^3$ CFU per ml). During this peri d, the inhibition of cachectin by $F(ab')_2$ resulted in impr ved cardiac output, suggesting that cachectin is necessary t prov ke septic shock. The administration of $F(ab')_2$ alone with ut bacterial challenge did not significantly change blood pressure, cardiac output or hormone counter-regulatory release ver the 10-h monitoring period. Recovery from anaesthesia was uneventful, normal activity and food intake was resumed vernight and post-mortem examination was normal. The improved cardiovascular function in the antibody-treated bacteraemic animals cannot be attributed to differences in hydration status, because similar amounts of fluid were administered to all animals in accordance with a predetermined protocol (data not shown).

During sepsis, the systemic release of catabolic stress hormones in part mediates the maintenance of cardiovascular tone and mobilization of host energy stores[15]. We observed persistent increases in circulating adrenaline, noradrenaline, and glucagon in all animals succumbing to bacterial challenge (Table 2), but early pre-treatment with antibodies blunted the magnitude of later (8 h) counter-regulatory hormone responses in all survivors. We have previously shown that the infusion of recombinant cachectin produces hypotension and diminished cardiac output despite endogenous catecholamine production (which would n rmally increase cardiac output)[10]. As antibody administration is ass ciated with improved cardiac output, the present data als suggest that sepsis-associated myocardial depression is due in part to cachectin.

It appears then that the normal injurious h st responses elicited by verwhelming bacteraemia can be prevented if the effects f cachectin are blocked. The death of the infected host could result from an verexpression of cachectin during a normally beneficial immune response, somewhat analogous to anaphylactic shock, during which protective immune responses are capable of inducing shock and death. It is probable that the provocative toxicity of cachectin arises in part from a direct effect on normal tissues, and in part from the release of other humoral factors. Complement activation, release of the interleukins, interferons and platelet-activating factor, and the induction of other cytokines would be expected to amplify and broaden the range of host responses[16-19].

Experimental models of endotoxaemia and sepsis perhaps differ from the indolent and p. gressive syndrome of multipl system organ failure in human patients, but cachectin has been implicated in human infections[20,21]. Levels of cachectin are frequently elevated during acute menigicoccal sepsis, when those patients with the highest cachectin levels die[22]. The prophylactic use of anti-cachectin antibodies in patients at high risk for overwhelming infection could protect against septic shock. Further experiments are needed to determine whether the administration of anti-cachectin antibodies during persisting infection or indolent sepsis will be beneficial.

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γ Interferon, CD8+ T cells and antibodies required for immunity to malaria sporozoites

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This study was designed t test the hypothesis that T-cell effector mechanisms are required for protective immunity to malaria sporozoites. Administration of neutralizing monoclonal antibodies against γ interferon (γIFN) to immune hosts, reversed sterile immunity to sporoz ite challenge, by allowing the growth of